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Basic Investigation

Influence of acupuncture on neural movement function in rats with middle cerebral artery occlusion-A randomized controlled trial

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Abstract

OBJECTIVES: To observe recovery in movement function in rats with middle cerebral artery occlusion (MCAO) after acupuncture treatment.

METHODS: According to the randomized and controlled principle 1384 rats were divided into the basic control group (including the normal, sham, model control, model without intervention, Nimodipine, and para-Renzhong groups) and the acupuncture group (including the Neiguan (PC 6), Weizhong (BL 40), Sanyinjiao (SP 6), Chize (LU 5), Renzhong (GV 6) and non-acupoint groups). MCAO was modeled by Zea-longa's thread ligation and rats with scores of 1-3, as assessed by Zausinger's six-point method, were used in this study. Moreover, in the acupuncture group each acupoint was set with 12 different parameters by the orthogonal intersection method, resulting in 78 groups with 18 rats per group. The rats were treated by acupuncture once every 12 h for a total of six sessions and neurobehavioral scores were measured after each session. The neurobehavioral scores were compared by one-way ANOVA using the statistical software SPSS 17.0.

RESULTS: After acupuncture therapy the mean neurobehavioral scores in MCAO rats increased gradually at each time point with a significant difference among the six scores, but with no significant differences between the fourth (48 h) and the fifth score (60 h), and between the fifth (60 h) and the sixth (72 h) score ($P > 0.05$).

CONCLUSIONS: MCAO rats gradually recovered movement function over multiple acupuncture sessions. After the fouth acupuncture session (48 h), the neurobehavioral scores of rats with cerebral infarction remained stable. Acupuncture treatment had a reliable curative effect on movement function in cerebral infarction rats.

Key words: MCAO Rat; Acupunctural Therapy; Neurobehavioral/Neural Ethological Score; One-way Analysis of Variance (One-way ANOVA)

INTRODUCTION

Stroke, namely "wind-stroke", refers to acute cerebrovascular disease, characterized by the symptoms of sudden fainting, unconsciousness, paralysis of limbs, facial palsy and difficulty in speaking. Stroke is a common intractable disease with the significant "three highs", high morbidity, high disability and high mortality. It is divided into ischemic stroke and hemorrhagic stroke. Stroke is a great threat to human health and life, brings about great pain to patients, and creates significant family and social burden. Therefore, it is very important to improve methods for prevention and treatment of stroke, so as to reduce the morbidity, disability and mortality.

Acupuncture is an effective method of treating cerebral infarction; previous research has indicated that the acupuncture, possibly through improving blood flow, affecting the microstructure, and regulating protein and gene expression[1-4], can improve ethological scores in the rats. Therefore, in this paper neurological behavior was assessed six times using Zausinger's six-point method[5] and the scores were statistically analyzed by one-way ANOVA, so as to preliminarily study the recovery of movement function in cerebral infarction rats treated by acupuncture.

MATERIALS AND METHODS

Animals and grouping

1384 adult, male, SPF Wister rats, weighing 250 - 300g, were provided by the Laboratory Animal Center of the Chinese Academy of Medical Sciences (Beijing, China), with the certificate for animal epidemic prevention No. 050275.

According to randomized controlled principles the rats were divided into a basic control group (including normal control group, sham group, model control group, model group without intervention, Nimodipine group, para-Renzhong group) and an acupuncture group (including Neiguan (PC 6) group, Weizhong (BL 40) group, Chize (LU 5) group, Sanyinjiao (SP 6) group, Renzhong (DU 6) group and non-acupoint group). Moreover, in the acupuncture groups for each acupoint or needling site 12 different combined parameters were set by the orthogonal intersection method. There were 18 rats in each group, with 78 groups in total. Among them, 15 model rats were excluded after MCAO and 1369 rats were enrolled.

Duplication and Judgment of Successful MCAO Rat Model

Duplication of MCAO rat model: The MCAO model was duplicated with Zea-longa's thread ligation method [5]. In brief, the rats were fasted for 12 h with free access to water and anesthetized by intraperitoneal injection of 10% hydration chlorine aldehyde (350 mg/kg). The rats were then fixed in the dorsal position on the surgery board. After local disinfection, an incision of 2-2.5 cm was made in the middle of the neck on the left side, the thyroids on both sides were separated, the triangle area between the sternocleidomastoid muscle and the sternohyoid muscle on the left was exposed, the left common carotid artery and external carotid artery were bluntly separated, and the external carotid artery was ligated with 0 suture line. The carotid artery was clipped in the arterial bifurcation/fork and near the heart with a small artery clamp and a small hole near the heart was pierced with a 1 mL syringe needle. Fishing-line 0.26 mm in diameter was slowly inserted into the internal carotid until resistance was met, with an intracranial depth of 18-20 mm. Blood flow in the left middle cerebral artery was blocked by plugging the artery with fishing line. After successful carotid artery ligation, the cephalic artery and pinhole were ligated and another small artery clamp was opened. At last the incision was sterilized and sutured layer by layer.

Judgment of successful MCAO rat model: After the modeled rats woke, their neurological function was assessed by the Zausinger six-point method [6] and rats with scores of 4 and 5 were excluded. The standards used to obtain the Zausinger six-point score were as follows: 0 score, the rat could not spontaneously walk; 1 score, the rat rotated towards the side opposite to the lesion with free walking; 2 score, the rat rotated towards the side opposite to the lesion when its tail was seized; 3 score, the resistance to the lateral pressure was decreased in the side opposite to the lesion; 4 score, the rat could not unbend front paws or entire forelimb on the side opposite to the lesion; 5 score, the rat had no neurological function defect.

Acupuncture Intervention Methods

Selected acupoints and acupuncture methods: Neiguan (PC 6), Weizhong (BL 40), Chize (LU 5), Sanyinjiao (SP 6), Renzhong (GV 26) and non-acupoints were selected and needled with thrusting-lifting manipulation. On the basis of the acupoint location of commonly-used experimental animals in the *Experimental Acupuncture Science* [7], Neiguan was located at the forefoot, between the ulna and the radius, about 3 mm from the wrist, perpendicularly needled 3 mm in depth; Weizhong was located on the right rear indenta-

tion of the knee-joint, perpendicularly needled 3 mm; Chize was located on the indentation of the elbow horizontal grain near the radial side, perpendicularly needled 5 mm; Sanyinjiao was located on the medial side of the hind leg, 10 mm above the tip of the medial malleolus, perpendicularly needled 3 mm; Renzhong was located on the philtrum, 1 mm below the nasolabial sulcus, obliquely inserted towards the nasal septum; Non-acupoint, as a control, was located between iliac crest and ribs and 10 mm on the top of iliac crest on the affected side, obliquely inserted 2 mm.

Intervention methods for the groups: The acupuncture group: After successful modeling, in the corresponding period, the model rats were treated with the acupun ctu re intervention six times.

The model control group: After successful modeling, the model rats did not received any acupuncture. Cerebral blood flow and micro-circulation were first detected, then the rat's head was removed and the cerebral tissue was taken for light microscopic examination and testing of infarction rate.

The model without intervention group: After successful modeling, the model rats were held without any acupuncture intervention six times.

The normal control group: The normal rats did not receive the acupuncture intervention, but were also held six times in the experimental period.

The sham group: Except that the fishing thread was not inserted, the operations were the same as the acupuncture group, and the neurobehavioral scores assessed by the Zausinger six-point method in all the rats of the sham group were 5.

The Nimodipine group: After successful modeling, the model rats were treated with intraperitoneal injection of Nimodipine at 0.15 mg/kg, once per day.

The para-Renzhong group: After modeling, in the corresponding period, the model rats were needled 2 mm in depth with perpendicular insertion on the left side (affected side) of philtrum line, 5 mm lateral to the Renzhong (GV 26).

Needles selected: "Huatuo" brand filiform needles made in Suzhou Medical Products Factory, 1.5 inches in length and 0.32 mm in diameter were used.

Intervention Parameters: Except for the normal control group, the sham group, the model control group, the model without intervention group, and the Nimodipine group, acupuncture intervention was performed on the remaining groups according to the orthogonal intersection method of acupuncture parameters [2 factors (frequency and time), 3 levels of frequency (180 Hz, 120 Hz, 60 Hz) and 4 levels of time (5 s, 60 s, 120 s, 180 s), see Table 1].

Table 1 12 Different combined acupuncture parameters

Acu. Para.	2	3	4	5	6	7	8	9	10	11	12	13
Acu. Fre.(Hz)	60	120	180	60	120	180	60	120	180	60	120	180
Acu. Time(s)	5	60	180	60	180	5	180	5	60	120	120	120

Note: In this paper, influences of the acupunctural frequency and time on the acupuncture effect were mainly discussed; Parameter 1was used for the needle-keeping group, so, it was not discussed here.

Intervention time: One hour after the model rats were completely awake, acupuncture was given for the first time. It was repeated once every 12 h, for a total of six times.

Statistical method: In order to test if recovery of movement function was induced by the acupuncture treatment in MCAO model rats, SPSS 17.0 statistical software was used for one-way analysis of variance (one-way-ANOVA) for the neuroethological scores over the six ratings.

RESULTS

It can be seen from Table 2 that the means of the neurological function scores from the first to the sixth rating gradually increased, indicating that with each additional session of acupuncture therapy, neurological function progressively recovered in the rats with cerebral infarction.

For Levene's homogeneity of variance test the F= 26.454, and P=0.000, showing that the variance of the groups was significantly different.

The table of one-way ANOVA showed that the sum of squares between groups S_A=325.063, degree of freedom $df_A = 5$, the sum of mean-square between groups MS_A= 65.013; F=107.773, and P<0.05, indicating that there was a significant difference in the six observed rating scores.

On the condition of non-homogeneity of variance, the two relatively robust statistics of Welch and Brown-Forsythe were chosen. These tests still showed a significant difference in the six neurological function ratings (P<0.05).

The outcome of *Dunnett's C* multiple comparisons (Table 3) showed a significant difference between the neurological function scores at the six time points in MCAO rats after acupuncture therapy. When multiple comparisons were made, there were no differences between the fourth (48 h) and the fifth (60 h), and the fifth (60 h) and sixth (72 h), P>0.05, indicating that after the fourth acupuncture session (48 h), the state of the rats with focal cerebral infarction are basically stable.

After acupuncture therapy, the means of the neurobehavioral scores increased at each time point, with significant differences between the scores at the six time points. When multiple comparisons were made, there was no difference between the fourth (48 h), the fifth (60 h), and the sixth (72 h) (P>0.05).

Table 2 Statistical Table of Neurological Function Scores

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
1.00	1369	2.2126	.86058	.02326	2.1669	2.2582	1.00	5.00
2.00	1369	2.5172	.77131	.02085	2.4763	2.5581	.00	5.00
3.00	1369	2.6012	.74743	.02020	2.5615	2.6408	.00	5.00
4.00	1369	2.6947	.74357	.02010	2.6552	2.7341	.00	5.00
5.00	1369	2.7473	.75923	.02052	2.7070	2.7875	.00	5.00
6.00	1369	2.8232	.77196	.02086	2.7823	2.8642	.00	5.00
Total	8214	2.5993	.80153	.00884	2.5820	2.6167	.00	5.00

Table 3 Test of Multiple Comparisons

(I)Score	(J)Score	Mean Difference (I-J)	Std. Error	95%Confidence Interval	
				Lower Bound	Upper Bound
1.00	2.00	-.30460*	.03123	-.3937	-.2155
	3.00	-.38860*	.03081	-.4765	-.3007
	4.00	-.48210*	.03074	-.5698	-.3944
	5.00	-.53470*	.03102	-.6232	-.4462
	6.00	-.61066*	.03125	-.6998	-.5215
2.00	1.00	.30460*	.03123	.2155	.3937
	3.00	-.08400*	.02903	-.1668	-.0012
	4.00	.17750*	.02896	-.2601	-.0949
	5.00	-.23009*	.02925	-.3136	-.1466
	6.00	-.30606*	.02949	-.3902	-.2219
3.00	1.00	.38860*	.03081	.3007	.4765
	2.00	.08400*	.02903	.0012	.1668
	4.00	-.09350*	.02849	-.1748	-.0122
	5.00	-.14609*	.02879	-.2283	-.0639
	6.00	-.22206*	.02904	-.3049	-.1392
4.00	1.00	.48210*	.03074	.3944	.5698
	2.00	.17750*	.02896	.0949	.2601
	3.00	.09350*	.02849	.0122	.1748
	5.00	-.05259	.02872	-.1346	.0294
	6.00	-.12856*	.02897	-.2112	-.0459
5.00	1.00	.53470*	.03102	.4462	.6232
	2.00	.23009*	.02925	.1466	.3136
	3.00	.14609*	.02879	.0639	.2283
	4.00	.05259	.02872	-.0294	.1346
	6.00	-.07597	.02926	-.1595	.0075
6.00	1.00	.61066*	.03125	.5215	.6998
	2.00	.30606*	.02949	.2219	.3902
	3.00	.22206*	.02904	.1392	.3049
	4.00	.12856*	.02897	.0459	.2112
	5.00	.07597	.02926	-.0075	.1595

DISCUSSION

Neurobehavioral score is one of the major indices used for diagnosis and evaluation of cerebral infarction. It is widely used for clinical and experimental studies, because it is a simple and non-invasive method that al-

lows for continuous observation. Neurobehavioral score is mainly applied in the form of the rating scale used to measure functional changes. In the light of different functions, the rating scale can be divided into the movement function deficit scale, cognitive dysfunction scale, mood rating scale and pain ethological rating scale, etc. The movement function score is mainly applied for evaluation of cerebrovascular disease models, which mostly induce damage in body movement function, therefore, myodynamia and movement, etc. can be assessed. The Zausinger method is the acknowledged standard for rating neurological movement impairments in MCAO model rats and is frequently used to assess movement function after stroke.

Our research group has rich experience in the study of treatment of cerebral infarction by the Xingnao Kaiqiao method. In the present study, one-way ANOVA analysis of changes in neurological scores over six time points after acupuncture therapy showed that rats with MCAO gradually recovered movement function after repeated acupuncture sessions. In addition, our results demonstrated that after the fourth acupuncture session (48 h), the state of the rats with focal cerebral infarction was basically stable, indicating that acupuncture treatment had a good effect on neurological movement function in rats with cerebral infarction. At the same time, the analysis of the neurobehavioral data also indicated that the experimental data obtained is better and lays a foundation for the future research of the topic.

The Zausinger six-point method overall reflects the degree of damage and therapeutic effect of acupuncture, but in this experiment it was found that the grading method had a small gradient of scoring criteria and was unable to clearly show the degree of damage to the central nervous system. Some researchers have discovered this problem and put forward corresponding improvement measures. For example, Wei E Q combined the hang test and climb board to quantitatively evaluate the neurological symptoms of focal cerebral ischemia mice[8], but with the shortcomings of larger individual differences, need of special apparatus, and a heavy workload in processing data. Therefore, development of evaluative methods with convenience, high sensitivity and reliability is expected and would aid further research on the effects of acupuncture after stroke[9].

CONCLUSIONS

With repeated acupuncture, MCAO rats gradually recovered function. After the fourth acupuncture session (48 h), the state of rats with cerebral infarction was stable. The acupuncture treatment has a good reliable curative effect on neurological movement function in the cerebral infarction rat.

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